[82-1b. Pirimicarb Tech. Subchronic - feeding dog/1968]

9/20/1996

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SUPPLEMENTAL DATA EVALUATION REPORT
(HED DOC.#s: 001725, 001726 & 011637)

STUDY TYPE: 90-day Feeding Study - Dog

OPPTS NUMBER: 870.3151 **OPP GUIDELINE NUMBER:** 82-1(b)

DP BARCODE: D215390 **SUBMISSION CODE:** None

PC CODE: 106101 **TOX. CHEM NO:** 359C

MRID NO.: 43641001 (previously 00113439 and 00113440)

TEST MATERIAL: PP062; Pirimicarb

SYNONYMS: 2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate

REPORT NUMBER: CTL/P/4593 incorporating CTL/R/241 and CTL/R/248 (previously IHR/241 and IHR/248)

SPONSOR: Imperial Chemical Industries

TESTING FACILITY: Zeneca Central Toxicology Lab.*
Alderley Park, Macclesfield,
Cheshire, UK

*Previously known as
Imperial Chemical Industries
Industrial Hygiene Res. Labs.,

TITLE OF REPORT: Subchronic Studies with Pirimicarb (PP062) in the Beagle Dog

(Ninety-day Oral Toxicity of PP062 - Beagle Dogs, Part 1)

and

(Oral toxicity of PP062-Beagle Dogs, Part 2)

AUTHOR: M C E Hodge

STUDY COMPLETED: March 1968 and December 1968

EXECUTIVE SUMMARY: In a two part dietary subchronic study (MRID# 43641001), 4 beagle dogs/sex/dose received in Part 1 either 0, 4, 10 or 25 mg/kg/day and in Part 2 either 0, 0.4, 1.8 or 4 mg/kg/day of PP062 (94% a.i.). Half of the dogs/dose in Part 1 were sacrificed after 90 days other half was allowed 28 days for recovery. The 0, 0.4 and 1.8 mg/kg/day dose groups were sacrificed after 90 days but the 4 mg/kg/day dose group was continued on the test diet for 180 days. Part 2 was limited to an assessment of the hematological changes noted in Part 1. These studies were done prior to implementation of GLP Guidelines, therefore, do not fall under purview of either GLP or Quality Assurance requirements.

Test chemical effects on bone marrow cytology were evident at 4 mg/kg/day in both Parts 1 and 2. In particular in Part 2, **proerythroblasts** were increased in both males/females at 30, 60 and 90 days having values of 3.7/3.4, 4.2/5.4 and 6.6/4.9 counts, respectively, compared to counts of 0.5/0.8 in the controls verifying the increases noted in Part 1 at all dose levels. Other indications of bone marrow cytology and hematology also indicated effects (see DER for details). During the course of the study one high-dose male and female and one mid-dose female developed macrocytic anemia. High-dose male died and the death was attributable to anemia and this dog exhibited erythropoietic hyperplasia. There was **decreased body weight** in males (about 4%, $P < 0.05$) at 25 mg/kg/day. The bone marrow effects were said by the study author to be indicative of "a compound-dependent, hemolytic anemia of the 'penicillin type'." **The Systemic Toxicity LOEL is 4 mg/kg/day, based on hematopoietic effects. The Systemic Toxicity NOEL is 1.8 mg/kg/day.**

Part I. Plasma ChE was inhibited in 10 and 25 mg/kg/day dose males/females by approximately 24.8%/1.92% and 34.5%/18.5%, respectively, at 12 weeks; plasma ChE was significantly inhibited in both sexes by second week. RBC AChE activity was inhibited in mid- and high-dose males (16.4% and 27.7%, respectively) and high-dose females (21%). In Part 2, plasma ChE and RBC AChE were not affected. **The LOEL is 10 mg/kg/day, based on plasma ChE and RBC AChE inhibition. The NOEL is 4 mg/kg/day.**

The studies combined are classified as **Acceptable** and **satisfy** the regulatory requirements (82-1b) for subchronic toxicity study in dogs.

A. **MATERIALS:**

1. **Test compound:** PP062. Description - a colorless odorless crystalline solid, Batch # - not given, Purity - 94%.

2. **Test animals:** Species: canines, Strain: Beagles (inbred), Age: not given; probably fully mature considering initial mean body weights of 15 kg for males and 11.6 kg for females, Source: Alderly Park, Cheshire, England.

B. **STUDY DESIGN:**

Part 1 of the study was designed to determine the NOEL of PP062 in the diet of Beagle dogs when fed for 90 days. RBC, plasma and brain ChE inhibition and bone marrow erythroid:myeloid ratio were the main parameters used for estimating the NOEL. Part 2 of the study was done to verify the NOEL for hematological changes seen in Part 1.

1. **Animal assignment**

Animals were assigned to the following test groups:

TABLE 1. ANIMAL ASSIGNMENT

TEST GROUP	DOSE (MG/KG)	DURATION (DAYS) OF DOSING	NO. OF ANIMALS	
			♂	♀
PART 1				
I (Cont)	0	90	4	4
II (LTD)	4	90	4	4
III (MTD)	10	90	4	4
IV (HTD)	25	90	4	4
PART 2				
I (Cont)	0	90	4	4
II (LTD)	0.4	90	4	4
III (MTD)	1.8	90	4	4
IV (HTD)	4.0	180	4	4

In Part 1, all dogs received the above doses for 90 days. Half the dogs were continued for an additional 28 days undosed. In Part 2, all dogs received doses for 90 days, except the group IV which received treatment for 180 days.

2. **Diet preparation**

The compound was suspended by ball milling in Dispersol OG and an appropriate amount was added daily to canine diet. The frequency of sample preparation was not specified. Samples of treated food were not analyzed for stability and concentration.

3. Animals received 45 g meat preparation ("Kennomeat", Scottish Animal Products Ltd.) and 226 g dry pelleted diet ("Kennel Kernals", James & Co., Hungerford) daily and water ad

libitum. For Part 2 of the study feeding schedule was not provided.

4. **Statistics** - Original submission did not include any statistical analysis of the data. In the current submission, the data were analyzed by ANOVA and covariance using the GLM procedure in SAS (1989). Least-square means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-square mean and control group least-squares mean. The differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

5. The studies were done in 1968 i.e., long before the implementation of GLP Guidelines; therefore, do not fall under purview of either GLP or Quality Assurance requirements.

C. **METHODS AND RESULTS:**

1. **Observations:**

One high-dose (25 mg/kg) male (# 27) became ill after 10 weeks, lost weight, became lethargic and developed urinary incontinence and severe anemia and died during the 11th week of study. Necropsy revealed abdominal ascites, a heavy nematode infestation, and congestion of the thymus, spleen and liver. The death of this dog was considered treatment-related. In Part 2, one control male (#11) and one top dose female (# 29; 4 mg/kg) exhibited lethargy, loss of appetite, weight loss, pyrexia, slight dehydration and bloody stools (female) during the weeks 4 and 13 of the study. Both animals recovered in a few days, though the male showed a recurrence of symptoms on two occasions. At necropsy both animals showed hemorrhagic cystitis, polypoid in the female, with congested abdominal lymph nodes. In addition, lung inflammation in male and mammary congestion in females were observed. These clinical signs were also observed periodically in the kennels. The author considered the above clinical signs observed in Part 2 of the study were unrelated to treatment. TB-1 concurs with the authors conclusions.

2. **Body weight**

All animals were weighed weekly for 13 weeks.

Results - Table 1 presents adjusted mean bodyweights of males and females at selected intervals. Adjusted mean bodyweights of males receiving 25 mg/kg/day were significantly different from controls in most weeks between 5 and 14 and are considered treatment-related. The adjusted mean bodyweight of males slightly decreased from week 14 but the differences were not significant. The female adjusted bodyweights were not affected due to treatment.

In Part 2, the adjusted bodyweights of males and females were not affected.

TABLE 1. ADJUSTED BODYWEIGHTS (KG)1

INTERVAL (WEEKS)	MALES DOSE (MG/KG/DAY)				FEMALES DOSE (MG/KG/DAY)			
	0	4	10	25	0	4	10	25
5	15.22	15.23	15.00	**14.96***	11.67	12.15*	11.91	11.68
10	15.34	15.25	15.14	**14.68***	11.93	12.45*	12.22	11.92
15	15.39	15.14	14.80	14.40	12.19	13.60	11.85	12.90
18	15.49	15.37	15.00	14.24	12.46	13.21	12.11	12.47

1 Copied from report CTL/P/4593, P 85 - 90
N = 4 from week 1 to 14 and all others N = 2
* = $P \leq 0.05$

3. **Food consumption and compound intake**

Food Consumption per se was not determined but assumed that food offered was consumed and as well as the compound in the food.

4. **Ophthalmological examination**

Ophthalmological examination was not performed.

5. **Blood was collected** before treatment and at termination (90 days) for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined. In Part 2, hemoglobin, PCV, MCHC, MCH, reticulocyte count, platelets, clotting function and serum iron levels were determined pre-treatment and at monthly intervals.

a. **Hematology**

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
X	Blood clotting measurements	X	Mean cell diameter (MCD)
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

Results - Part I. During the course of the study **one high-dose male (#27) and female (#30) and one mid-dose female (#21) developed macrocytic anemia.** The high-dose (25 mg/kg/day) male died during the 11th week of study. Bone marrow of the dead dog exhibited erythropoietic hyperplasia. At termination an increase in the number of circulating erythroblasts were seen in these dogs. Table 2 presents adjusted (covariate adjustment was based on the separate sex pre-experimental group means) hematology parameters from Part 1 of study. In the high-dose males/females, the hemoglobin (Hgb) and packed cell volume (HCT) decreased 9.6%/10.5% and 11.5%/9.9%, respectively; and as expected, the mean cell diameter increased 3.7%/5.1%, when compared to the controls. **Mean cell diameter increased in all treated males and females, but reached statistical significance in only female receiving 25 mg/kg/day, and is considered treatment-related in the high-dose only. Reticulocyte counts, expressed as % of total RBC, increased in all treated groups which suggest a slight stimulation of bone marrow (range 1 - 4) and were considered treatment-related in all dose levels.** Lymphocytosis was observed in both sexes and was statistically significant ($P \leq 0.05$) in mid-dose males and high-dose females; the response was dose-related only in females. Elevated lymphocyte numbers reflect an response to foreign antigens. **Lymphocytosis in both sexes at all doses are considered treatment-related.**

TABLE 2. SELECTED, ADJUSTED HEMATOLOGY PARAMETERS

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES DOSE (MG/KG/DAY)			
	0	4	10	25	0	4	10	25
HGB (g/100ml)	16.6	17.3	16.3	15.0	17.2	16.8	14.6	15.4
HCT (%)	49.4	50.8	48.6	43.7	50.4	48.5	42.7	45.4
MCD (μm)	6.00	6.09	6.03	6.22	6.03	6.06	6.14	**6.34***
Recticulocytes (% of RBC)	0.3	2.3	2.2	0.9	1.0	2.5	3.5	3.5
Lymphocytes ($X10^3$/cmm)	3.37	4.92	**5.52***	5.01	3.47	4.72	5.38	**5.98***
Monocytes ($X10^3$/cmm)	0.41	0.43	0.11	0.61	0.50	0.45	**0.06***	0.24

1 Copied from Report Table 7, P 112 - 127
N = 4; includes data from main and recovery phase of the study
* $P \leq 0.05$

Table 3 presents bone-marrow cytology and includes the **myeloid** (myeloblasts, promyeloctes, promyeloctes - neutrophils and eosinophils, metamyelocytes - neutrophils and eosinophils), **erythroid** (proerythroblasts, early normoblasts, intermediate normoblasts, late normoblasts and megaloblasts) and **other cell** (monocytes, plasmacytes and lymphocytes) series of the main and recovery phase of the study (Part 1).

Erthyroid series generally increased in the males and was statistically significant in the high-dose males, however in females no general trend was observed. Megaloblasts, the precursors of red cells, which depend on vitamin B_{12} and/or folate for maturation into proerythroblasts were significantly ($P \leq 0.05$) higher in high-dose males and low and mid-dose females, compared to controls. Table 4 presents bone-marrow cytology from part 2 of the study. The means for various bone-marrow cells were not affected. When the means were adjusted, the mean number of megaloblasts in 4 mg/kg/day group males and females increased at all time points and the differences were significant ($P \leq 0.01$). The adjusted (covariate adjusted based on sex pre-experimental group means) mean number of megaloblasts in males/females at 30, 60 and 90 days evaluation were 3.7/3.4, 4.2/5.4 and 6.6/4.9, respectively, compared to 0.5 to 0.8 of controls (data not shown in table). **The number of megaloblats at all dose levels in Part 1 and high-dose males and females in Part 2 are considered treatment-related.**

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TABLE 3. BONE MARROW CYTOLOGY1

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES (MG/KG/DAY)			
	0	4	10	25	0	4	10	25
Myeloblasts	0.6 ± 0.3	0.8 ± 0.4	**1.6* ± 0.8**	0.9 ± 0.4	0.7 ± 0.4	1.3 ± 0.5	**1.4* ± 0.3**	0.8 ± 0.0
Promyelocytes	1.2 ± 0.9	1.7 ± 0.7	**2.8* ± 1.8**	1.5 ± 0.9	1.8 ± 0.3	1.9 ± 0.6	1.0 ± 0.7	1.8 ± 1.1
Promyelocytes - neutrophils	9.9 ± 3.9	8.8 ± 1.6	7.3 ± 3.3	7.0 ± 1.8	7.5 ± 2.4	5.9 ± 1.6	4.7 ± 1.8	6.8 ± 1.9
Promyelocytes - eosinophils	2.2 ± 0.4	1.6 ± 1.2	1.8 ± 1.2	1.4 ± 0.8	2.1 ± 1.2	1.5 ± 0.3	1.9 ± 1.4	1.6 ± 0.2
Metamyelocytes	3.3 ± 2.0	3.5 ± 0.4	3.5 ± 3.0	3.7 ± 2.6	2.7 ± 1.2	1.9 ± 0.7	3.0 ± 1.9	3.7 ± 1.3
Metamyelocytes - neutrophils	20.7 ± 8.5	13.8 ± 1.8	20.3 ± 4.4	19.3 ± 10.9	23.0 ± 0.8	19.5 ± 6.8	13.4 ± 9.8	19.8 ± 1.7
Metamyelocytes - eosinophils	1.7 ± 1.6	1.7 ± 1.3	1.3 ± 0.6	1.1 ± 0.5	1.9 ± 1.2	1.8 ± 1.2	1.0 ± 0.9	1.9 ± 0.3
TOTAL MYELOIDS	39.6	31.9	38.6	34.9	39.7	33.8	26.4	36.4
Lymphocytes	2.3 ± 1.0	1.9 ± 0.4	4.4 ± 3.0	1.8 ± 1.7	5.7 ± 2.8	**2.3* ± 1.5**	**2.8* ± 2.9**	**2.3* ± 0.9**
Proerythroblasts	3.0 ± 0.6	3.4 ± 0.9	3.3 ± 1.2	**5.7* ± 2.8**	2.8 ± 0.8	2.8 ± 0.7	**5.0* ± 1.5**	4.9 ± 1.0
Early Normoblast	5.0 ± 1.4	6.9 ± 1.4	6.2 ± 1.7	**14.4* ± 9.6**	4.2 ± 1.1	6.1 ± 1.1	7.2 ± 2.8	7.1 ± 0.6
Int. Normoblast	22.4 ± 5.3	18.4 ± 5.1	17.1 ± 2.4	**10.5* ± 8.1**	20.0 ± 1.7	14.0 ± 4.3	18.1 ± 7.6	12.0 ± 3.9
Late Normoblast	23.3 ± 0.6	17.8 ± 2.6	20.8 ± 2.7	**9.9* ± 7.2**	25.3 ± 2.4	**11.2* ± 2.8**	17.8 ± 11.2	21.3 ± 9.4
Megaloblast	3.0 ± 1.3	18.4 ± 5.6	9.5 ± 6.5	**21.5* ± 7.2**	1.5 ± 0.4	**28.5** ± 7.6**	**21.0* ± 21.5**	14.5 ± 9.9
TOTAL ERYTHROIDS	56.7	64.9	56.9	63.8	53.8	62.6	80.1	59.8
M:E	0.7:1.0	0.49:1.0	0.68:1.0	0.54:1.0	0.73:1.0	0.54:1.0	0.33:1.0	0.6:1.0

1 Data extracted from Report Table 8, P 128 - 135
* = $P \leq 0.05$, ** = $P \leq 0.001$

TABLE 4. BONE MARROW CYTOLOGY ON DAY 90[1]

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES (MG/KG/DAY)			
	0	0.4	1.8	4.0	0	0.4	1.8	4.0
Myeloblasts	1.0 ± 0.6	0.9 ± 0.8	0.7 ± 0.2	1.0 ± 0.3	1.2 ± 0.6	0.9 ± 0.5	1.3 ± 0.5	1.3 ± 0.5
Promyelocytes	1.0 ± 0.4	0.8 ± 0.5	0.8 ± 0.2	0.5 ± 0.3	1.0 ± 0.8	0.7 ± 0.3	0.8 ± 0.4	1.0 ± 1.2
Promyelocytes - neutrophils	2.8 ± 1.7	3.2 ± 1.5	1.9 ± 0.4	4.6 ± 1.5	3.9 ± 0.4	3.7 ± 1.0	4.2 ± 1.1	4.7 ± 0.8
Promyelocytes - eosinophils	1.1 ± 0.7	1.3 ± 0.3	1.3 ± 0.7	1.3 ± 0.5	1.5 ± 0.9	1.8 ± 1.4	1.9 ± 1.1	1.7 ± 0.1
Metamyelocytes	2.5 ± 0.5	2.0 ± 1.1	2.3 ± 0.6	2.4 ± 1.4	1.9 ± 0.4	2.6 ± 0.9	2.5 ± 1.1	3.3 ± 1.4
Band Forms	6.8 ± 2.2	5.0 ± 1.6	8.9 ± 4.2	9.3 ± 2.7	8.7 ± 2.0	8.6 ± 1.2	8.8 ± 2.5	9.7 ± 1.0
Metamyelocytes - neutrophils	18.6 ± 5.8	16.6 ± 3.2	16.6 ± 2.3	12.1 ± 5.1	13.6 ± 5.9	13.9 ± 2.8	12.1 ± 2.6	11.0 ± 3.6
Metamyelocytes - eosinophils	2.4 ± 1.4	2.4 ± 0.8	1.5 ± 0.8	1.4 ± 1.3	2.2 ± 0.9	2.1 ± 0.6	1.5 ± 0.7	1.3 ± 0.5
TOTAL MYELOIDS	36.1	32.2	34.0	32.6	34.0	34.3	33.1	34.0
Lymphocytes	1.8 ± 1.7	1.1 ± 0.6	0.5 ± 0.5	1.5 ± 0.3	0.6 ± 0.8	1.2 ± 0.7	1.5 ± 0.9	1.0 ± 0.9
Proerythroblasts	1.3 ± 0.9	1.8 ± 0.6	1.5 ± 0.7	1.8 ± 0.8	1.1 ± 0.5	1.4 ± 0.6	1.5 ± 0.2	2.2 ± 0.5
Early Normoblast	2.2 ± 0.9	2.9 ± 1.4	2.8 ± 1.5	3.9 ± 1.5	1.8 ± 1.4	2.5 ± 0.4	2.5 ± 1.2	3.2 ± 0.9
Int. Normoblast	10.0 ± 3.6	13.3 ± 3.5	12.4 ± 0.6	9.3 ± 3.0	9.3 ± 3.2	13.4 ± 1.6	10.7 ± 1.4	11.5 ± 1.7
Late Normoblast	45.0 ± 9.2	45.7 ± 3.4	44.9 ± 3.8	43.2 ± 8.5	51.0 ± 4.6	45.8 ± 7.0	47.3 ± 3.1	42.3 ± 5.6
Megaloblast	0.6 ± 0.3	1.4 ± 0.8	2.8 ± 0.5	6.8 ± 2.8	0.5 ± 0.4	1.0 ± 0.3	1.4 ± 1.2	4.8 ± 3.4
TOTAL ERYTHROIDS	59.1	65.1	64.4	65.0	63.7	64.1	63.4	64.0
M:E	0.6:1.0	0.49:1.0	0.53;1.0	0.53:1.0	0.53:1.0	0.53:1.0	0.52:1.0	0.53:1.0

1 Data extracted from Report Table 5, P 188 - 221

Myeloid series (cells). In both males and females the myeloid and other cell types were not affected. Promyelocytes in 10 mg/kg/day males and myeloblasts in mid-dose males and females increased significantly ($P \leq 0.05$), however, the increase lacked dose-response, therefore, considered not be toxicologically significant.

Myeloid/Erythroid (M:E) ratio of bone marrow at terminal sacrifice are presented in Table 3 and 90 day sampling from Part 2 in Table 4. These ratios were calculated by the reviewer. In Part 1 of the study, the M:E ratios generally decreased but no clear trend was evident. The report indicated that one mid-dose female and high-dose male had about 3 fold decrease in the erythroid mass which resulted in the M:E ratio in these groups. The author described these bone marrow changes were indicative of delayed maturation and the decreased M:E ratio should be described as "a compound-dependent, hemolytic anemia of the 'penicillin type'." In Part 2 (Table 4), M:E were not affected.

Based on the hematology/bone marrow changes, the NOEL = 1.8 mg/kg/day and the LOEL = 4.0 mg/kg/day.

b. **Clinical Chemistry**

X	Electrolytes:	X	Other:
	Calcium		Albumin
	Chloride		Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	Enzymes		Total bilirubin
X	Alkaline phosphatase (ALK)		Total serum Protein (TP)
X	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Serum folate		
X	Vitamin B_{12}		
X	Serum iron		

i) Serum folate, Vitamin B_{12} and Iron:

Serum folate/vitamin B_{12} and iron levels were determined on

control, one mid-dose (#21♀) and high-dose dogs during week 12 of the study.

Results: The serum folate (♂/♀ control - 11.3 ± 4.1/4.8 ± 0.5 and 25 mg/kg/day - 7.6 ± 0.5/7.7 ± 4.7) and vitamin B_{12} (♂/♀ control - 416 ± 85/391 ± 27 and 25 mg/kg/day - 355 ± 58/540 ± 136) levels were not different than the controls. **In males, the mean serum iron levels in the controls and 25 mg/kg/day Group were 138.9 ± 16.2 and 98.1 ± 4.4 (mg/100ml), respectively, and the difference was significantly different (P ≤ 0.05).** In females, iron serum levels were not affected. In Part 2 of the study, the **adjusted (by analysis of variance, separate for males and females) mean serum iron levels of females in the 4.0 mg/kg/day group at 30, 60 and 90 day evaluations decreased 22.3%, 45.8% and 42.4%, respectively, compared to controls; the 60 and 90 day observations were significantly different (P ≤ 0.05 to 0.01).** **Decreased iron levels are considered treatment-related in 25 mg/kg/day males.**

ii) Cholinesterase

Table 5 presents adjusted (by analysis of covariance on pre-treatment values, separately for males and females) mean plasma and RBC cholinesterase values from Part 1 of the study at selected intervals. **Plasma ChE was inhibited significantly (P ≤ 0.05 to 0.01) for males and females in the 10 and 25 mg/kg/day groups during most of the study.** Plasma ChE inhibition reached significant levels by the end of week one and remained inhibited most of the study period. By week 12 (at the end of the study) for males receiving 4, 10, or 25 mg/kg/day pirimicarb, plasma ChE was inhibited 1.4%, 24.8% or 34.5%, compared to controls; and was significant in the two highest doses. In females, at the same doses and at the same time point, the ChE inhibition was not statistically significant. **RBC** AChE appeared inhibited initially in about 6 weeks, the dose-response and the trends were generally similar to plasma, and reached significant levels by week 12. In males, at 4, 10 or 25 mg/kg/day, the % AChE inhibition was 10.8, 16.4 or 27.2, respectively, when compared to controls. In females, at the same doses, the % inhibition was 4.8, 9.6 or 21, respectively, when compared to controls. **RBC AChE inhibition was statistically significant in males receiving 10 and 25 mg/kg/day and in females receiving 25 mg/kg/day only.** Plasma ChE and RBC AChE levels returned to normal or control levels within a week following cessation of dosing. **Brain** AChE levels were determined on one or two animals at week 12 and after 4 week of recovery; these levels

approximated the controls. **The NOEL = 4 and LEL = 10 mg/kg/day.**

TABLE 5. MEAN PLASMA ChE AND RBC AChE ACTIVITY (μmol/ml/min)1

INTERVAL (WEEKS)	MALES DOSE (MG/KG/DAY)				FEMALES DOSE (MG/KG/DAY)			
	0	4	10	25	0	4	10	25
Pre-treatment-Plasma	2.23 ± 0.20	2.07 ± 0.13	2.35 ± 0.28	2.18 ± 0.14	1.87 ± 0.16	2.21 ± 0.18	2.39 ± 0.27	2.14 ± 0.08
RBC	1.75 ± 0.18	1.51 ± 0.13	1.52 ± 0.16	1.63 ± 0.28	1.82 ± 0.28	1.79 ± 0.08	2.08 ± 0.29	1.50 ± 0.19
1 Plasma	2.25	2.07	**1.83****	**1.61****	2.17	2.14	**1.76****	**1.57****
RBC	1.26	1.31	1.33	1.42	1.56	1.65	1.77	1.61
2 Plasma	2.15	2.16	**1.83***	**1.50****	2.18	2.02	**1.71***	**1.82***
RBC	1.25	1.19	1.25	1.24	1.58	1.58	1.71	1.50
6 Plasma	2.48	2.67	2.11	**1.98***	2.59	2.32	1.96	**1.98***
RBC	1.73	1.70	1.58	1.44	2.06	2.03	1.86	1.80
8 Plasma	2.61	2.52	2.52	1.90	2.11	2.19	2.09	2.54
RBC	1.73	1.69	1.65	1.35	1.62	1.98	2.09	1.69
12 Plasma	2.78	2.74	**2.09***	**1.82****	2.60	2.90	2.55	2.12
RBC	1.95	1.74	**1.63***	**1.42****	2.09	1.99	1.89	**1.65****
Recovery 1 Plasma	2.92	2.94	2.83	**3.69****	2.83	2.67	2.83	3.50
RBC	1.76	1.75	1.45	1.84	1.84	2.28	1.72	2.20
Recovery 4 Plasma	2.68	2.59	2.57	2.84	2.80	2.71	3.07	2.79
RBC	1.76	1.46	1.54	1.57	1.89	1.92	1.88	1.77

1 Data extracted from Report Tables 2 and 3, P 91 - 98
* $P \leq 0.05$, ** $P \leq 0.01$
Values reported are adjusted means

6. **Liver Function**

The bromsulphalein (B.S.P.) retention test, as a means of assessing hepatic function, is currently out of vogue because of many draw backs inherent in the test. The B.S.P. retention values were increased in all treated groups in Part 1; were significantly ($P \leq 0.05$) elevated in low and high-dose females. In females, at the 4, 10 or 25 mg/kg/day, the post-treatment adjusted retention time was 2.91, 2.53 or 2.98 minutes, respectively, compared to the 1.74 for the controls. In males, at the 0, 4, 10 or 25 mg/kg/day, the adjusted BSP values were 2.09, 2.24, 2.56 or 2.72 minutes, respectively. The B.S.P retention times are within the expected range (< 5% retention at 30 minutes). B.S.P. retention times were not done in Part 2 of the study. **The test is unreliable to assess liver function.**

7. **Urinalysis**

Urinalysis was done pre- test and at terminal sacrifice from all animals (Part 1 only). **Urine collection procedure or conditions of collection were not described.** The CHECKED (X) parameters were examined.

X		X	
	Appearance	X	Glucose
	Volume		Ketones
X	Specific gravity	X	Bilirubin
X	pH		Blood
	Sediment (microscopic)		Nitrate
X	Protein		Urobilinogen

Results - Urinalysis showed no changes attributable to treatment. Urine sp. gr. of treated males and females ranged 1.02 to 1.04 vs 1.02 to 1.03 of controls (normal 1.015 to 1.040). Urine pH of males and females ranged from 5.9 to 6.7 vs 6.0 to 6.3 for controls (normal 5.5 to 7.0).

8. **Sacrifice and Pathology**

In Part 1, two animals per Group were sacrificed on schedule and the remaining dogs were observed for 28 days and then euthanized. All animals were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. In addition, brain tissue (cerebrum, cerebellum and pons cerebelli), spinal cord and sciatic nerve were taken from four males and four females in Groups I and IV. In Part 2, all necropsy procedures listed in Part 1 were evaluated

at the scheduled sacrifice.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta	XX	Brain +
X	Salivary glands	XX	Heart		Periph. nerve
	Esophagus	X	Bone marrow		Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen		Eyes (optic n.)
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		XX	Adrenal gland
	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder		Mammary gland
	Rectum	XX	Testes +		Parathyroids ++
XX	Liver +	X	Epididymides	XX	Thyroids ++
	Gall bladder		Prostate	Other	
	Pancreas		Seminal vesicle		Bone
Respiratory		X	Ovaries +		Skeletal muscle
	Trachea	X	Uterus		Skin
XX	Lung				All gross lesions and masses
	Nose				
	Pharynx				
	Larynx				

a. **Organ weight** - The adjusted (covariate adjustment was based on sex pre-treatment group means) organ to body wt. ratios were not affected. There was an apparent reduction (34%) in adrenal/bodyweight in 25 mg/kg/day female, compared to controls and was explained by the study author as due to a large adrenal in control dog #5 (0.0266 vs 0.0106). In Part 2, the adjusted liver/bodyweight ratios of 0.4, 1.8 and 4.0 mg pirimicarb/kg/day treated males decreased 14.5, 19.3 and 15.4%, respectively, compared to controls. The 0.4 and 1.8 mg/kg/day liver/bodyweight was significant ($P \leq 0.05$). No statistical analysis was performed for the 4.0 mg/kg/day group because these animals were sacrificed 90 days later. The study author concluded that the organ/bodyweight changes were not treatment-related since there was no dose-response and the differences appear to have risen as a result of two high control values (animal #s 2 and 4). We concur with the study Author's conclusions.

b. **Gross pathology** - Dogs necropsied (10 mg/kg-#21 & 25 mg/kg-#27) due to severe anemia revealed hemopoiesis of spleen and lymph nodes. Dog #30 was necropsied after a recovery period of 28 days showed no abnormality. **All dogs were light to heavily infested with intestinal**

parasites. Dogs in Part 2 of the study exhibited no gross abnormalities.

c. **Microscopic pathology** - A higher incidence of focal inflammatory lesions in the liver and reactive changes in lymph nodes were reported observed in the pirimicarb treated animals (Part 1). Two dogs (#s 21 & 27) which were sacrificed due to severe anemia, exhibited hemapoiesis of the spleen and lymph nodes. The anemic dog (#30) which was maintained for a further 28 days, the histological abnormalities observed above were reversed. Dogs in Part 2 of the study maintained for 6 months on 4 mg/kg/day did not show any histological changes seen in Part 1, except bone marrow had an increased number of proerythroblasts. In addition delayed red cell maturation (megaloblasts) at all time points and normal population of precursor cells (stem cells) were observed. These finding support a LOEL of 4.0 mg/kg/day and NOEL of 1.8 mg/kg/day for hematological effects.

D. **DISCUSSION:**

The studies were conducted in 1968 prior to implementation of GLP guidelines and therefore, does not meet the current guideline criteria. The primary reason for reevaluating these studies are to update old DERs and extract pertinent information which could be used to establish the **systemic and ChE NOEL and LOEL in a nonrodent species. None of the studies individually is adequate to establish the toxicity end-points. However, in combination these studies provide some of the required information. Further, this DER reflects the corrected terminology for "megaloblasts" as "proerythroblasts".** The sponsor rectified this terminology in the past and the Agency has accepted the terminology (HED Doc. # 001323 dated August 1, 1980).

In a two part dietary subchronic study (MRID# 43641001), 4 beagle dogs/sex/dose received in Part 1 either 0, 4, 10 or 25 mg/kg/day and in Part 2 either 0, 1.8 or 4 mg/kg/day of PP062 (94% a.i.). Half of the dogs/dose in Part 1 were sacrificed after 90 days except and the other were allowed 28 days for recovery. The 0 and 1.8 mg/kg/day dose groups were sacrificed after 90 days but the 4 mg/kg/day dose group was continued on the test diet for 180 days. Part 2 was limited to an assessment of the hematological changes noted in Part 1. These studies were done prior to implementation of GLP Guidelines, therefore, do not fall under purview of either GLP or Quality Assurance requirements.

Test chemical effects on bone marrow cytology were evident at 4 mg/kg/day in both Parts 1 and 2. In particular in Part 2, **proerythroblasts** were increased in both males/females at 30, 60 and 90 days having values of 3.7/3.4, 4.2/5.4 and 6.6/4.9 counts, respectively, compared to counts of 0.5/0.8 in the controls verifying the increases noted in Part 1 at all dose levels. Other indications of bone marrow cytology also indicated effects (see DER for details). During the course of the study one high-dose male and a female and one mid-dose female developed macrocytic anemia. High-dose male died and the death was attributable to anemia and this dog exhibited erythropoietic hyperplasia. There was **decreased body weight** in males (about 4%, $P < 0.05$) at 25 mg/kg/day. The bone marrow effects were said by the study author to be indicative of to be associated with a "a compound-dependent, hemolytic anemia of the 'penicillin type'." **The Systemic Toxicity LOEL is 4 mg/kg/day, based on hematopoietic effects. The Systemic Toxicity NOEL is 1.8 mg/kg/day.**

Part I. Plasma ChE was inhibited in 10 and 25 mg/kg/day dose males/females by approximately 24.8%/1.92% and 34.5%/18.5%, respectively, at 12 weeks; plasma ChE was significantly inhibited in both sexes by second week. RBC AChE activity was inhibited in mid- and high-dose males (16.4% and 27.7%, respectively) and high-dose females (21%). In Part 2, plasma ChE and RBC AChE were not affected. **The LOEL is 10 mg/kg/day, based on plasma ChE and RBC AChE inhibition. The NOEL is 4 mg/kg/day.**

The two studies combined are classified as **Acceptable** and **satisfy** the regulatory requirements (82-1b) for subchronic toxicity study in dogs.

Note: Megaloblasts have been changed to proerythroblasts to correct for classification errors cited in the HED Doc. #001323 dated August 1, 1980.

REDDY\D215390\Pirimor/9/26/95
Final: 08/07/96

Sign-off date:	09/20/96
DP Barcode:	d215390
HED DOC Number:	012061
Toxicology Branch:	tb1